REMARKS

The Specification has been amended in order to comply with the requirements for nucleotide and/or amino acid sequence disclosures as set forth in 37 C.F.R. 1.821-1.825. Sequence listing identifiers have been inserted that reference the Sequence Listing submitted concurrently to BOX SEQUENCE, P.O. Box 2327, Arlington, VA 22202. Marked-up and clean versions of the individual Substitute Sheets are enclosed herewith.

Applicants further concurrently submit to Box Sequence a computer readable form (CRF) copy and a paper copy of the "Sequence Listing" in order to comply with the requirements for nucleotide and/or amino acid sequence disclosures as set forth in 37 C.F.R. 1.821-1.825. Applicants submit that the content of the paper and computer readable copies of the Sequence Listing are the same and do not go beyond the disclosure of the application as originally filed.

Applicants submit that this application is in condition for substantive examination, which action is respectfully requested.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to BOX PCT, Commissioner for Patents, Washington, DC 20231, on February 10, 2003.

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induction of programmed cell death. Particular peptides found to be capable of inducing programmed cell death include a sequence of human $eIF4G_{569-580}$, wheat $eIF4G_{62-73}$ and human $eIF4E\text{-}BP(1\&2)_{51-62}$ and derivatives and fragments thereof. Numbering according to Accession numbers AF104913, M95746, NM_004095 and NM_004096 respectively.

Thus the peptides of use in the present invention include the sequences;

human $eIF4G_{569-580}$, KKRYDREFLLGF [SEQ ID NO: 1]

wheat $eIF4G_{62-73}$ RVRYSRDQLLDL [SEQ ID NO: 2] and,

human $eIF4E\text{-}BP(1\&2)_{51-60}$ RIIYDRKFL(L/M) [SEQ ID NO: 3], and variants or derivatives thereof. A consensus may be derived from the above three sequences.

Thus, in a further aspect the present invention provides use of a peptide comprising a sequence:

YxxxxLØ [SEQ ID NO: 4]

wherein x is a variable amino acid and Ø is Leu, Met or Phe;

or a fragment or derivative thereof in therapy, more particularly for the induction of programmed cell death.

Alternatively the peptide may comprise the sequence:

(K/R)xxYxxx(F/Q)L(L/M) [SEQ ID NO: 5]

It is to be understood that "K/R" refers to an amino acid which is either lysine (K) or arginine (R), "x" may be any of the 20 amino acids or may be a synthetic or unnatural amino acid, "F/Q" refers to an amino acid which is either phenylalamine (F) or glutamine (Q) and "L/M" refers to an amino acid which is either leucine (L) or

methionine (M). The remainder of the sequence is understood to relate to the standard single letter symbol for amino acids.

Particular sequences may include KKRYDREFLLGF [SEQ ID NO: 1] (human eIF4G$_{413-424}$), RVRYSRDQLLDL [SEQ ID NO: 2] (wheat eIF4G$_{62-73}$) and RIIYDRKFL(L/M) [SEQ ID NO: 3] (human eIF4E-BP$_{51-60}$).

The invention also relates to the use of fragments and derivatives of these peptides. Fragments are defined herein as any portion of the peptides described that substantially retain the activity of the parent peptide. Derivatives are defined as any modified forms of said peptides which also substantially retain the activity of the parent peptide. Such derivatives may take the form of amino acid substitutions which may be in the form of like for like eg. a polar amino acid residue for another polar residue or like for non-like eg. substitution of a polar amino acid residue for a non-polar residue as discussed in more detail below.

Thus, the present invention further provides derivatives of the sequences disclosed above for use in the induction of cell death.

Replacement amino acid residues may be selected from the residues of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The replacement amino acid residue

and homologous and non-homologous substitution is defined using these classes. Thus, homologous substitution is used to refer to substitution from within the same class, whereas non-homologous substitution refers to substitution from a different class or by an unnatural amino acid.

In general, the term "peptide" refers to a molecular chain of amino acids with the defined biological activity. If required, it may be modified *in vivo* and/or *in vitro*, for example, by glycosylation, myristoylation, amidation, carboxybolation or phosphorylation. Thus inter alia peptides, oligopeptides and polypeptides are included. The peptides disclosed herein may be obtained, for example, by synthetic or recombinant techniques known in the art.

The term also extends to cover, for example, polypeptides which contain any of the above disclosed sequences and, in particular, wherein biological activity, that is, the polypeptide is capable of binding to eIF4E protein, is retained. Typically the length of the peptides of the present invention are between 7 - 25 amino acids in length, more preferably 10 - 20 amino acids in length.

In a further aspect the present invention provides use of a peptide comprising sequence:

YxxxxLØ [SEQ ID NO: 4] wherein x is a variable amino acid and Ø is Leu, Met or Phe;

or fragment or derivate thereof in the manufacture of a medicament for therapy, more particularly for inducing cell death.

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In particular, the peptide is used to induce the cell death in tumour cells.

In yet a further aspect, the present invention provides a polynucleotide fragment encoding a peptide comprising sequence:

YxxxxLØ [SEQ ID NO: 4] wherein x is a variable amino acid and Ø is Leu, Met or Phe.

"Polynucleotide fragment" as used herein refers to polymeric form of nucleotides of any length, both to ribonucleic acid sequence and to deoxyribonucleic acid sequences. In principal, this term refers to the primary structure of the molecule, thus this term includes double stranded and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

As described above, the presence of a peptide comprising the above sequences can induce programmed cell death (apoptosis) in mammalian cells. The peptides of the present invention therefore have utility in treating diseases associated with undesirable cell proliferation/neoplasia. In particular the peptides have utility as anticancer or antitumour agents. Therefore, it may be desirable to direct the peptides to the site of action ie. the tumour. Thus, in the case of peptides, they may be conjugated to or associated with cell and/or tumour targeting agents, or in the case of the polynucleotide fragments provided as an expression cassette which comprises a polynucleotide sequence which encodes any of the above disclosed peptides, and a tumour-specific

elevated translation of growth related mRNAs, which are normally translationally repressed (19). In order to study directly the role of eIF4E in cell transformation, a series of experiments were carried out.

Human eIF4G$_{(413-424)}$ was conjugated to Penetratin, a known cell membrane translocation peptide of sequence RQIKIWFQNRRMKWKK [SEQ ID NO: 6] (see patent EP485578). Description of its synthesis and coupling to other peptides may be found in US Patent 5,888,762. The human eIF4G$_{(413-424)}$-Penetratin conjugate was found to bind recombinant human eIF4E *in vitro* (see Figure 4). Surprisingly, wheat eIF4E$_{(62-73)}$ bound to and pulled down more recombinant human eIF4F *in vitro* than human eIF4G$_{(569-580)}$ did (see Figure 5). It was also observed that recombinant human 4E-BP1 competed with either human eIF4G$_{(569-580)}$ or wheat eIF4G$_{(62-73)}$ for binding of recombinant human eIF4E *in vitro* (see Figure 6).

Wheat eIF4G$_{(62-73)}$ was found to inhibit cap-dependent translation initiation, but not cap-independent translation initiation *in vitro* (see Figure 7). However, inhibition of cap dependent translation by eIF4G peptides was not detected in cultured mammalian cells. Furthermore, no inhibition of general translation by peptides from eIF4G or 4E-BP was detected in cultured mammalian cells.

Human eIF4G$_{(569-580)}$-Penetratin exhibited a cytotoxic or cytostatic effect on selected cell lines (HaCaT cells, no effect observed with short treatment (<24h with 20μM) but treatment of 60h serum starved cells began to die within 15 minutes of peptide treatment. Furthermore, human eIF4G$_{(413-}$

CLAIMS

1. Use of an eIF4E binding agent in therapy.

2. Use according to claim 1 for the induction of programmed cell death.

3. Use according to either of claims 1 or 2 wherein the agent is a peptide or peptidemimetic.

4. Use according to claim 3 wherein said peptide comprises the sequence:
YxxxxLØ [SEQ ID NO: 4]
wherein x is a variable amino acid and Ø is Leu, Met or Phe;
or a fragment or derivative thereof.

5. Use according to claim 4 wherein said peptide comprises the sequence:
(K/R)xxYxxx(F/Q)L(L/M) [SEQ ID NO: 5].

6. Use according to claim 5 wherein said peptide comprises the sequence:
KKRYDREFLLGF [SEQ ID NO: 1],
RVRYSDQLLDL [SEQ ID NO: 2], or
RIIYDRKL(L/M) [SEQ ID NO: 3].

7. Use according to any of claims 3 - 6 wherein said peptide is 7 -25 amino acids in length.

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8. Use of a peptide comprising sequence:
YxxxxLØ [SEQ ID NO: 4] wherein x is a variable amino acid and Ø is Leu, Met or Phe;
or fragment or derivate thereof in the manufacture of a medicament for therapy, more particularly for inducing cell death.

9. Use according to claim 8 wherein the medicament is used to induce cell death in tumour cells.

10. Use of a polynucleotide fragment encoding a peptide comprising sequence:
YxxxxLØ [SEQ ID NO: 4] wherein x is a variable amino acid and Ø is Leu, Met or Phe.